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review

Interleukin 2: from immunostimulation to immunoregulation and back again

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Interleukin 2 (IL-2) was one of the first cytokines to be discovered. However, the complex role of IL-2 and its receptor in the regulation of immune responses is only now emerging. This review explores the various signals triggered by IL-2 and discusses their translation into biological function. A model is outlined that accommodates the seemingly contradictory functions of IL-2, and explains how one cytokine can be an essential T-cell growth and differentiation factor and yet also be indispensable to maintain peripheral tolerance.

Keywords: adaptive immunity; immune regulation; immune stimulation; interleukin 2; T lymphocyte

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Introduction

Interleukin 2 (IL-2) and its receptor (IL-2R) were the first cytokine and cytokine receptor to be cloned, more than 20 years ago (Leonard *et al*, 1984; Nikaido *et al*, 1984; Taniguchi *et al*, 1983). The first function attributed to IL-2 was a potent capacity to enhance *in vitro* T-cell proliferation and differentiation (Gillis *et al*, 1978; Gillis & Smith, 1977; Morgan *et al*, 1976; Smith, 1988), and it was therefore originally named T-cell growth factor (TCGF). In line with its *in vitro* function, IL-2 was also assumed to have a crucial role *in vivo* during antigen-driven clonal expansion of T cells. As IL-2 is mainly produced by activated T cells and, in particular, by activated $CD4^+$ T-helper cells, at least part of their 'helper' function for $CD8^+$ T cells was attributed to IL-2 (Keene & Forman, 1982). Subsequent to these initial descriptions of the function of IL-2, numerous studies have highlighted many more seemingly contradictory functions of this cytokine (Fig 1). With respect to immune-enhancing functions, IL-2 has a role in supporting proliferation (Bamford *et al*, 1994; Gillis *et al*, 1978; Gillis & Smith, 1977; Morgan *et al*, 1976; Smith, 1988; Smith *et al*, 1980) and survival (Blattman *et al*, 2003) of T cells, and differentiation of naive T cells into effector and memory cells (Cho *et al*, 2007; Kamimura & Bevan, 2007; Ke *et al*, 1998). Recent evidence indicates that IL-2 is also an important factor that allows the generation of memory T cells, which are able to undergo secondary expansion when they re-encounter an antigen (Bachmann *et al*, 2007; Williams *et al*, 2006). Furthermore, IL-2 has the ability to overcome the proliferation block of anergic cells generated *in vitro* (Powell *et al*, 1998; Schwartz, 1996) and, in certain situations, also *in vivo* (Kündig *et al*, 1996).

In opposition to these immune-enhancing functions, IL-2 can promote activation-induced cell death (AICD) of T cells (Dai *et al*, 1999; Lenardo, 1991; Refaeli *et al*, 1998; Zheng *et al*, 1998) and was therefore implicated in downregulating antigen-specific T-cell numbers after the clonal expansion phase of an immune response (Khoruts *et al*, 1998; Kneitz *et al*, 1995; Ku *et al*, 2000; Refaeli *et al*, 1998; Van Parijs *et al*, 1997).

IL-2 also has anti-inflammatory properties, as do other pro-inflammatory cytokines, such as interferon-γ (IFNγ; Bachmann & Kopf, 2002). In a similar process to IFNγ—which exerts anti-inflammatory properties by suppressing T-helper 17 cells—IL-2 can constrain IL-17 production (Laurence *et al*, 2007), and exert its immunosuppressive function by stimulating the generation and homeostasis of regulatory T cells (T_{REG}). Indeed, IL-2 is a non-redundant factor for the *in vivo* homeostasis of T_{REG}, which constitute a fundamental part of immunological self-tolerance and immune regulation (D'Cruz & Klein, 2005; Fontenot *et al*, 2005; Klebb *et al*, 1996; Papiernik *et al*, 1998; Suzuki *et al*, 1999; Wolf *et al*, 2001).

The aim of this review is to summarize and integrate the diverse functions of IL-2 with a particular emphasis on its *in vivo* roles as assessed in experimental murine models.

IL-2 and IL-15: similar receptors, different function

The high-affinity receptor for IL-2 is a heterotrimeric membrane protein complex consisting of an IL-2-specific α-subunit (IL-2Rα, CD25), a β-subunit (IL-2Rβ, CD122) and the common cytokine receptor γ-chain (γc, CD132). The IL-2Rα chain alone binds to IL-2 with low affinity (dissociation constant (K_d) = ~10^{-8} M) and has a short cytoplasmic tail that is not involved in recruitment of cytoplasmic signal transduction molecules. In combination with the IL-2Rβ and γc chains, the affinity of the receptor for IL-2 is increased by three orders of magnitude (K_d = ~10^{-11} M). In addition, the cytoplasmic domains of the IL-2Rβ and γc chains are responsible for

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signal transduction through activation of the Janus kinase 3 (JAK3)/signal transducer and activator of transcription 5 (STAT5) and AKT-dependent signalling pathways, and the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways, respectively (reviewed in Kovanen & Leonard, 2004; Ma *et al*, 2006; Waldmann, 2006). The receptor for IL-15 has a similar structure to that of IL-2R: they share the IL-2/15Rβ (CD122) and γc (CD132) chains. It is the receptor α-chains that are unique for the respective receptors and that confer specificity. However, unlike the IL-2Rα chain, which binds to IL-2 with low affinity (10^{-8} M), the IL-15Rα chain alone binds to IL-15 with high affinity (10^{-11} M), and is therefore well suited to capture IL-15 efficiently and to present it to other cells (see below). Because the signal transduction of both receptors is mediated through the cytoplasmic domains of the IL-2/15Rβ and γc chains, the receptors share JAK and STAT signalling pathways, and trigger phosphorylation of lymphocyte-specific protein tyrosine kinase (LCK) and spleen tyrosine kinase (SYK), which are members of the SRC family of protein tyrosine kinases. Both also induce expression of the anti-apoptotic B-cell lymphoma 2 (BCL-2) protein, and stimulate the PI3K–AKT and RAS/RAF MAPK pathways. This eventually leads to activation of c-FOS-containing and c-JUN-containing transcription-factor complexes (Miyazaki *et al*, 1995).

The signal transduction pathways shared between IL-2R and IL-15R might imply that they have similar functions and responses. Indeed, several functions are elicited by both cytokines: induction of T-cell proliferation, involvement in the differentiation of cytotoxic T lymphocytes, and generation, activation and persistence of natural killer (NK) cells, as well as stimulation of B-cell proliferation and immunoglobulin synthesis (reviewed in Waldmann *et al*, 2001). However, *in vivo* studies with knockout mice that lack IL-2, IL-15 or components of their respective receptors have shown that IL-2 and IL-15 also mediate a series of specific and non-overlapping functions. Mice that are deficient in IL-2 or in components of IL-2R exhibit a phenotype of pronounced and uncontrolled lymphoproliferation (Sadlack *et al*, 1993; Schorle *et al*, 1991; Suzuki *et al*, 1995; Willerford *et al*, 1995), which is linked to autoimmune disease, indicating that IL-2/IL-2R signalling is a crucial component of self-tolerance. IL-2/IL-2R signalling is required for the homeostasis of T_{REG} (D'Cruz & Klein, 2005; Fontenot *et al*, 2005; Klebb *et al*, 1996; Papiernik *et al*, 1998; Suzuki *et al*, 1999; Wolf *et al*, 2001), which in turn are crucial guards for maintaining self-tolerance and preventing immunopathology through the active modulation of adaptive immune responses—in particular, by inhibiting proliferation and differentiation of self-reactive T cells. Mice (and humans) that lack T_{REG} due to genetic defects—such as mutations in, or deletions of, the forkhead box P3 transcription factor (Foxp3), which is instructive for the development of T_{REG}—exhibit a phenotype similar to IL-2-deficient or IL-2R-deficient mice, which supports the view that IL-2 is crucial for the presence of T_{REG} (Brunkow *et al*, 2001; Fontenot *et al*, 2003). An additional role of IL-2 in controlling self-reactive T cells—and perhaps in downregulating T-cell responses—is mediated by its ability to promote AICD in T cells. AICD is induced in lymphocytes after repeated stimulation, and results from co-expression of the death receptor Fas (CD95) and its ligand FasL (CD95L; Brunner *et al*, 1995; Dhein *et al*, 1995; Ju *et al*, 1995; Krammer, 2000), as well as from repression of the anti-apoptotic protein FLIP (Refaeli *et al*, 1998). AICD is promoted in the presence of, and is dependent on, IL-2, as IL-2-deficient (Kneitz *et al*, 1995) and IL-2Rα-deficient T cells are resistant to it (Van Parijs *et al*, 1998). However, an important role for AICD in the downregulation of immune responses *in vivo* has been difficult to demonstrate. In fact, during many immune responses, T-cell frequencies decline after the antigen has largely been eliminated (and the T cells are therefore no longer activated by antigen). Even during chronic viral infections, IL-2 has been shown to enhance T-cell responses rather than blunt them (Bachmann *et al*, 2007; Blattman *et al*, 2003). Therefore, IL-2/IL-2R signalling is crucially involved in peripheral tolerance and immune regulation, whereas no comparable role can be attributed to IL-15. Instead, IL-15 seems to have a dominant role in the homeostasis of memory $CD8^+$ T cells, NK cells, NK T cells and subsets of intraepithelial lymphocytes (reviewed in Ma *et al*, 2006; Waldmann, 2006).

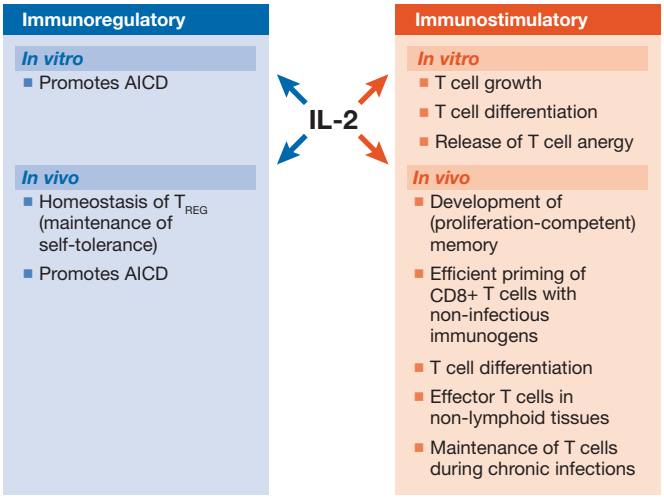

Fig 1 | *In vivo* and *in vitro* immunoregulatory and immunostimulatory functions of interleukin 2 (IL-2). AICD, activation-induced cell death; T_{REG}, regulatory T cell.

How do these two closely related cytokine receptors mediate specific and different signals *in vivo*? Several aspects contribute to the differences in the *in vivo* functions of IL-2 and IL-15: differential expression of the high-affinity cytokine receptors, distinct interactions with the respective receptors and some variability in signalling pathways. The α-chains of the IL-2 and IL-15 receptors are expressed in different cell types: IL-2Rα is expressed in activated T and B cells, whereas IL-15Rα is expressed in activated monocytes and dendritic cells (reviewed in Ma *et al*, 2006; Waldmann, 2006). IL-2 is secreted and upregulates expression of the IL-2Rα chain, binds to and stabilizes the trimeric complex IL-2Rα/IL-2/15Rβ/γc, and signals through JAK1/JAK3. By contrast, IL-15 is only secreted in small quantities and is mainly retained on the membrane bound to the IL-15Rα chain (Burkett *et al*, 2004). The membrane-bound IL-15/IL-15Rα complex can be recycled through endosomal vesicles and re-expressed on the cell surface, thereby allowing prolonged IL-15 presentation on activated dendritic cells and monocytes (Dubois *et al*, 2002). In contrast to IL-2—which binds in its soluble form to trimeric high-affinity receptors on the target cells—membrane-bound IL-15/IL-15Rα complexes bind to IL-2/15Rβ/γc receptors on the target cells even in the absence of IL-15Rα-chain expression.

Therefore, IL-15 signalling requires coordinated expression of IL-15 and IL-15Rα on the signal-providing cell (dendritic cell or monocyte), and is dependent on cell–cell contact between the signal-providing cell and the signal-receiving cell (NK cell or memory CD8$^+$ T cell; Burkett *et al*, 2004; Sandau *et al*, 2004). There are also indications that some distinct signalling pathways are involved in inducing proliferation through IL-2 or IL-15; while IL-15 induces proliferation by 12 kDa FK506-binding protein (FKBP12)-mediated activation of p70 S6 kinase, FKBP12 is not required for IL-2-induced proliferation (Dubois *et al*, 2003).

The temporal regulation of IL-2Rα-chain compared with IL-15Rα-chain expression might also be an important parameter that could directly translate into different signal-transcription kinetics. Indeed, IL-2 induces sustained PI3K/protein kinase B (PKB) signalling, whereas IL-15 induces only a transient signal, which correlates with sustained compared with transient expression of the respective receptors (Cornish *et al*, 2006). Furthermore, it is conceivable that many of the available IL-2/15Rβ/γc chains are pulled into a complex with the IL-2Rα chain, rendering these cells particularly sensitive to IL-2 but less so to IL-15. Therefore, activated T cells that transiently express IL-2Rα, or T_{REG} that constitutively express IL-2Rα, might preferentially receive signals through IL-2R that trigger their proliferation, differentiation and maintenance. By contrast, in steady-state conditions or after resolution of an immune response, memory T cells no longer express the IL-2Rα chain but have elevated levels of IL-2/15Rβ/γc chains instead, making them responsive to IL-15/IL-15Rα presented by dendritic cells or monocytes. This leads to a proliferative signal that allows the homoeostatic division, and therefore maintenance, of memory T-cell populations.

In vivo role of IL-2 in T-cell responses

IL-2 was believed initially to be required for the *in vivo* proliferation of T cells owing to its 'classic' function as a growth factor *in vitro*. Mice deficient in IL-2 or its receptor components were used to test this assumption, and, on many occasions, T-cell expansion after infection or immunization seemed to be reduced only marginally, if at all. Infection of IL-2-deficient mice with lymphocytic choriomeningitis virus (LCMV), vaccinia virus (Bachmann *et al*, 1995; Kündig *et al*, 1993) or vesicular stomatitis virus (VSV) (D'Souza & Lefrancois, 2003; D'Souza *et al*, 2002) showed normal expansion of CD8$^+$ T cells, and even peptide immunization led to normal expansion of CD8$^+$ T cells (Kramer *et al*, 1994), although some studies have shown reduced expansion and effector function of CD8$^+$ T cells after LCMV infection (Cousens *et al*, 1995; Utermohlen *et al*, 1994). Therefore, in many situations, IL-2 is a redundant growth factor *in vivo*. Furthermore, IL-15 can partly compensate for a lack of IL-2 in mediating T-cell proliferation, at least *in vitro* (Van Parjis *et al*, 1997). However, when the functionality of CD8$^+$ T cells derived from IL-2-deficient mice was assessed, varying degrees of impairment were observed. The effector function of virus-specific CD8$^+$ T cells after infection with vaccinia virus or LCMV seemed to be impaired only marginally, if at all (Bachmann *et al*, 1995; Kündig *et al*, 1993); in marked contrast, CD8$^+$ T cells that were primed by peptide immunization were dysfunctional (Kramer *et al*, 1994). These discrepancies could be the result of the differential induction of innate immune responses by infection as opposed to peptide immunization; while the former are potent inducers of innate responses, the latter show only limited activation of innate immunity, depending on the adjuvant used. Therefore, the plethora of cytokines induced upon infection might deliver differentiation factors, such as type 1 IFNs, IL-12 or IL-15, which could promote the development of effector functions in the absence of IL-2. As IL-2-deficient and IL-2Rα-deficient mice develop a lymphoproliferative disease and autoimmunity owing to the lack of T_{REG}, one might question the suitability of these models to determine quantitatively the role of IL-2 in regulating T-cell responses. It is likely that the proliferation of non-specific T cells to high levels might create an environment that also affects antigen-specific T-cell responses. Moreover, the lack of T_{REG} potentially favours the expansion of antigen-specific T cells. Because of this, it was important to re-address the role of IL-2 in the generation and maintenance of antigen-specific T-cell responses in healthy hosts with a normal T_{REG} population, and in the absence of ongoing lymphoproliferative disease. Several experiments were therefore conducted in which IL-2-deficient or IL-2Rα-deficient T cells that express transgenic (tg) T-cell receptors (TCRs) were adoptively transferred into hosts, and their responses to antigen stimulation were assessed *in vivo*. TCR tg T cells were either activated *in vitro* and then transferred into a lymphopenic RAG1-deficient host, or T-cell activation was performed directly *in vivo*. In both systems, IL-2-deficient or IL-2Rα-deficient T cells behaved similarly to control T cells with respect to the maintenance of cell numbers and secondary expansion after *in vivo* antigen challenge (Dai *et al*, 2000). One potential caveat to studies in which T cells are transferred into severely lymphopenic hosts is the non-specific activation and proliferation of the transferred T cells, which might affect the generation and maintenance of memory T cells. In subsequent studies, TCR tg CD8$^+$ T cells (wild type, IL-2-deficient or IL-2Rα-deficient) were transferred into normal hosts and their responses were compared after VSV infection. Initial cycling of the CD8$^+$ T cells was IL-2-independent, and CD8$^+$ T cells expanded normally in the absence of IL-2 or IL-2Rα expression in secondary lymphoid organs. However, the accumulation of CD8$^+$ T cells and their effector functions in peripheral organs were reduced, implying a role for IL-2 in homing to peripheral organs and/or in the accumulation and differentiation of effector cells within these organs (D'Souza & Lefrancois, 2003; D'Souza *et al*, 2002).

Another model used to investigate the role of IL-2 (and IL-15) in the induction and maintenance of T-cell responses in a normal environment is an IL-2/15Rβ-deficient mouse that expresses a tg form of IL-2/15Rβ exclusively in the thymus (Malek *et al*, 2000, 2001). In this model, peripheral T cells are non-responsive to IL-2 or IL-15, but the mice develop normal T_{REG} and do not show signs of abnormal lymphoproliferation or autoimmunity (Malek *et al*, 2002). T cells obtained from these tg mice showed a ~50% reduction in proliferation and a defect in acquisition of effector functions after *in vitro* activation (Malek *et al*, 2001). However, primary and secondary *in vivo* T-cell responses after infection with vaccinia virus, allogeneic skin grafting or *in vivo* anti-CD3 stimulation were relatively normal, indicating that both IL-2 and IL-15 are essentially dispensable for the *in vivo* expansion and differentiation of effector cells (Yu *et al*, 2003).

A new mouse model has been recently generated that allows the comparative analysis of IL-2Rα-deficient and IL-2Rα-sufficient CD8$^+$ T cells in a normal host environment without the use of adoptive transfer of TCR tg T cells. In this model, mixed bone-marrow chimeric mice are generated in which 50% of the haematopoietic system is derived from normal bone marrow and 50% is derived

from the bone marrow of IL-2Rα-deficient mice. Therefore, 50% of the CD8$^+$ T cells are IL-2Rα-deficient and cannot receive IL-2 signals, whereas the other 50% are normal and can receive them. These chimeric mice are healthy and do not develop a lymphoproliferative disorder or autoimmune disease. Two independent studies have analysed the CD8$^+$ T-cell response in these chimeric mice after LCMV infection, and have reached similar conclusions (Bachmann *et al*, 2007; Williams *et al*, 2006): the expansion, contraction and memory maintenance of LCMV-specific CD8$^+$ T cells was generally similar between IL-2Rα-deficient and IL-2Rα-sufficient CD8$^+$ T cells, although a fivefold reduction in numbers—but not in frequencies—was observed in one study (Bachmann *et al*, 2007). IL-2Rα-deficient and IL-2Rα-sufficient LCMV-specific CD8$^+$ T cells from secondary lymphoid organs, as well as from peripheral tissue, were also similar with respect to their effector functions, such as IFNγ and tumour necrosis factor-α (TNFα) secretion (Bachmann *et al*, 2007). Importantly, although the frequencies of IL-2Rα-deficient and IL-2Rα-sufficient LCMV-specific memory CD8$^+$ T cells were comparable, the IL-2Rα-deficient LCMV-specific CD8$^+$ T cells showed a marked impairment in their ability to undergo secondary expansion. This defect could be rescued if IL-2R signals were provided during the priming phase by administration of IL-2/anti-IL-2 monoclonal antibody complexes, which can signal through low-affinity IL-2R in the absence of IL-2Rα (Boyman *et al*, 2006; Williams *et al*, 2006). It therefore seems that IL-2 signals perceived during the priming period of CD8$^+$ T cells are essential for the programming of proliferation-competent memory CD8$^+$ T cells. However, impaired secondary proliferation could also be partly rescued with the same approach if the IL-2R signal was provided during the challenge phase.

These findings in CD8$^+$ T cells are in contrast to a recent report on the role of IL-2 signalling in CD4$^+$ T cells. IL-2R signalling seems to be a prerequisite for effective generation and maintenance of memory CD4$^+$ T cells (Dooms *et al*, 2007). However, autocrine IL-2 production by CD4$^+$ T cells does not seem to influence the extent of primary expansion, as adoptively transferred IL-2-deficient TCR tg CD4$^+$ T cells expanded to levels similar to those of their wild-type counterparts (Khoruts *et al*, 1998).

Additional studies in the mixed bone-marrow chimaeras showed that, although CD8$^+$ T-cell responses were efficiently induced after acute infection with live LCMV, immunization with non-replicating virus-like particles induced poor expansion of IL-2Rα-deficient CD8$^+$ T cells. This implies that, depending on the nature of the antigen and, perhaps, the level of innate immune activation or the duration of antigen availability, primary expansion or survival of activated cells can be largely IL-2-dependent or IL-2-independent (Bachmann *et al*, 2007).

As IL-2 is implicated in promoting AICD of T cells that are specific for self-antigens (reviewed in Abbas, 2003; Schimpl *et al*, 2002), it is conceivable that it might also have a role in down-regulating T-cell responses in the setting of chronic infections where antigens persist for prolonged periods of time. This possibility was also addressed in the mixed bone-marrow chimaeras. If these mice were infected with an LCMV strain that induces chronic infection, the IL-2Rα-deficient LCMV-specific CD8$^+$ T cells were rapidly deleted after an initial expansion, whereas IL-2Rα-sufficient LCMV-specific CD8$^+$ T cells were physically maintained (Bachmann *et al*, 2007). These results are supported by the previous finding that the injection of exogenous IL-2 during chronic infection sustained, rather than abrogated, T-cell responses (Blattman *et al*, 2003). Both results imply that IL-2 signals sustain CD8$^+$ T cells during chronic infection, rather than promote AICD as might have been predicted. In fact, during chronic infection, LCMV-specific CD8$^+$ T-cell responses rapidly cycle (Agnellini *et al*, 2007), and IL-2 might be required for the survival of at least some of these rapidly proliferating cells. Furthermore, in chronic viral infections, the virus-specific T-cell population is constantly replenished by the recruitment of new naive T cells (Vezys *et al*, 2006), and it is possible that IL-2 might have an important role in this process.

Recent data obtained after administration of antibody-potentiated IL-2 to naive mice indicate that strong IL-2 signals without deliberate immunization—and, hence, without specific TCR-triggered T-cell activation—induce the activation of T cells and their differentiation into functional central memory T cells (Cho *et al*, 2007; Kamimura & Bevan, 2007).

From these results, a picture emerges in which the diverse functions of IL-2 in the regulation of immune responses are dictated by the antigen kinetics of immunization or infection in relation to the dynamics of the T-cell response (Fig 2), the timing of IL-2 signals in relation to the immune response (Fig 3) and the extent of the activation of the innate immune system.

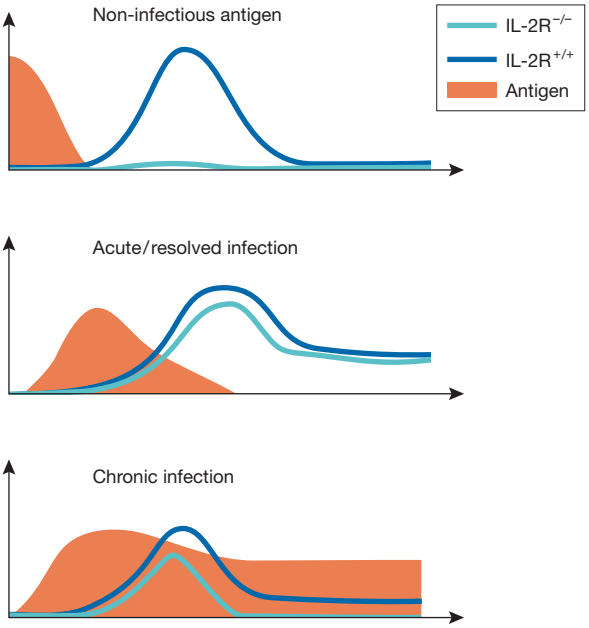

Fig 2 | Antigen kinetics and role of interleukin 2 in CD8$^+$ T-cell dynamics. Non-infectious antigens (such as non-replicating virus-like particles) require IL-2 signals for optimal expansion of CD8$^+$ T cells (Bachmann *et al*, 2007). Acute/resolved viral infections, such as lymphocytic choriomeningitis virus (LCMV) or vesicular stomatitis virus induce IL-2-independent primary CD8$^+$ T-cell responses (Bachmann *et al*, 2007; D'Souza & Lefrancois, 2003; D'Souza *et al*, 2002; Williams *et al*, 2006). During chronic viral infections (such as high doses of LCMV or murine cytomegalovirus) virus-specific CD8$^+$ T cells require IL-2 signals for their maintenance (Bachmann *et al*, 2007). The antigen load and availability are shown in red, whereas the antigen-specific CD8$^+$ T-cell responses are shown in green (IL-2Rα$^{-/-}$) or blue (wild type). IL-2, interleukin 2.

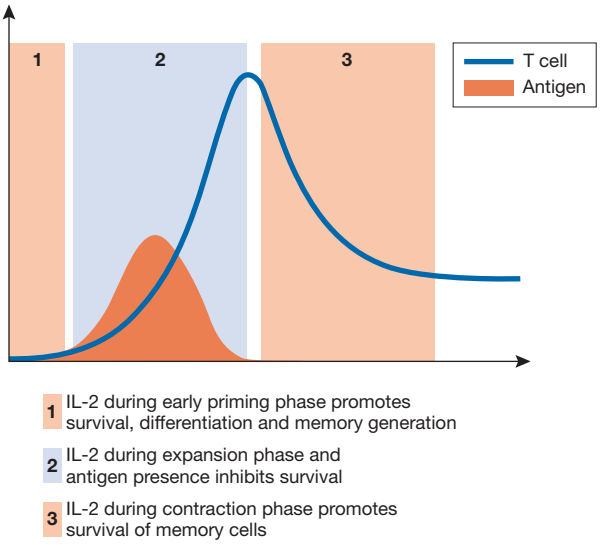

Fig 3 | The timing of interleukin 2 signals determines their impact on CD8$^+$ T-cell dynamics. Although IL-2 is dispensable for initiation of early cell cycling after T-cell receptor triggering, if it is provided to T cells during the early priming phase (phase 1), the differentiation, accumulation and memory formation of the T cells is improved. Also, when IL-2 is provided just after the peak expansion of T cells—when antigen is cleared and the T-cell frequencies decline (phase 3)—it can reduce the extent of the T-cell contraction (Blattman *et al*, 2003) and IL-2 promotes the survival of memory cells. By contrast, if IL-2 is provided during the expansion phase of a T-cell response (phase 2)—when T cells are activated and antigen is present—it is detrimental for the T cells as they will die by activation-induced cell death (Blattman *et al*, 2003). IL-2, interleukin 2.

IL-2, T-cell help, co-stimulation and anergic T cells

Multiple parameters affect the strength and duration of T-cell responses. Two factors, however, are particularly important: the presence of specific antigen (signal 1) and co-stimulation (signal 2). T cells that are primed in the absence of co-stimulation—that is, those that receive signal 1 in the absence of signal 2—are rendered anergic (Kündig *et al*, 1996; Schwartz, 2003). Such anergic T cells are characterized by an inability to produce IL-2 or to proliferate on antigenic stimulation, which is a phenotype that can be rescued by the addition of IL-2 (Powell *et al*, 1998; Schwartz, 1996). These characteristics are similar to those of T cells that have been primed in the absence of IL-2, which also show a profound impairment in their ability to undergo secondary expansion after re-encounter with antigen (Bachmann *et al*, 2007; Williams *et al*, 2006) that can be at least partly rescued by the addition of IL-2. Epigenetic modification of the IL-2 promoter on CD28 signalling might be the basis of the similarity of the two phenotypes. CD28 co-stimulation induces stable histone acetylation and loss of cytosine methylation of the IL-2 promoter, rendering it accessible to DNA-binding proteins (Thomas *et al*, 2005). Therefore, co-stimulation imprints the ability to produce IL-2 into the genome of T cells. The presence of IL-2 during priming might sustain this promoter modification, rendering the primed T cells fully proliferation competent.

It is interesting to note that cytotoxic T cells induced in the absence of helper T cells can exhibit a similar phenotype to T cells primed in the absence of co-stimulation or IL-2 signalling, as they are also proliferation-incompetent in several experimental systems (Bachmann *et al*, 2004, 2005; Janssen *et al*, 2003, 2005; Shedlock & Shen, 2003; Sun & Bevan, 2003) and show increased DNA methylation at the IL-2 promoter (Northrop *et al*, 2006). Because the functional deficits of memory CD8$^+$ T cells primed in the absence of CD28, IL-2 signalling or T-cell help are remarkably similar, it would be straightforward to propose that a lack of sufficient IL-2 is the underlying cause for all three phenotypes.

Concluding remarks

IL-2 is a cytokine that exhibits an impressive number of different functions largely dictated by the biological context in which it operates. It is pivotal for cellular activation, important for primary T-cell responses and essential for secondary T-cell responses. In addition, IL-2 has the key function of downregulating immune responses. Although IL-2 specifically promotes T-cell activation and proliferation of only those cells that have been stimulated by cognate antigenic interaction, downregulation of T-cell responses occurs non-specifically by facilitating a separate population of T_{REG}. As a consequence, a lack of IL-2 results in reduced antigen-specific T-cell responses as well as generalized non-specific T-cell activation. Harnessing our increased understanding of the biology of IL-2 might allow more selective targeting of specific IL-2 functions in the future.

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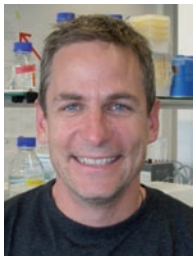

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